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*Published in:*  
Journal of Bacteriology

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*Document Version*  
Publisher's PDF, also known as Version of record

*Publication date:*  
1984

[Link to publication in University of Groningen/UMCG research database](#)

*Citation for published version (APA):*

van Waarde, A., & van Haastert, P. J. (1984). Transmethylation inhibitors decrease chemotactic sensitivity and delay cell aggregation in *Dictyostelium discoideum*. *Journal of Bacteriology*, 157(2), 368-74.

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# Transmethylation Inhibitors Decrease Chemotactic Sensitivity and Delay Cell Aggregation in *Dictyostelium discoideum*

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Received 18 July 1983/Accepted 21 November 1983

In *Dictyostelium discoideum*, extracellular cyclic AMP (cAMP) induces chemotaxis and cell aggregation. Suspensions of cAMP-sensitive cells respond to a cAMP pulse with a rapid, transient increase of protein carboxyl methylation. The transmethylation inhibitors cycloleucine, L-homocysteine thiolactone, and coformycin decrease chemotactic sensitivity and delay cell aggregation when administered in concentrations which do not influence cAMP binding to cell surface receptors or the activity of total phosphodiesterase. The ability of the drugs to inhibit chemotaxis could be correlated with their capacity to convert the initial transient positive response of carboxyl methylation to cAMP into a negative one. This suggests that both protein O-methyltransferase and protein methylesterase are activated after stimulation of aggregative cells with cAMP, the net effect being a transient, positive response of methylation. In the presence of a sufficiently large dose of inhibitor, methyltransferase is inhibited, whereas methylesterase activity is much less affected, so that a transient negative response of methylation to cAMP is observed. The slow, positive response of carboxyl methylation to cAMP which occurs ca. 2.5 to 5 min after stimulus administration is not affected by inhibitors of transmethylation. These results suggest that methylation reactions are involved in the chemotactic response of *D. discoideum* cells to cAMP.

Free living cells of the cellular slime mold *Dictyostelium discoideum* are able to locate their bacterial food by positive chemotaxis to folic acid (33). Lack of bacteria induces amoebae to aggregate. Cell aggregation is mediated by chemotaxis to cyclic AMP (cAMP) (19), which is detected by cell surface receptors (11, 12, 25, 26). cAMP is excreted in a pulsatile manner by the aggregation center, whereas individual amoebae show a capacity for signal relay (4). Addition of cAMP to a suspension of starved cells induces a transient increase of protein carboxyl methylation (27, 46; J. H. Nuske, Eur. J. Cell Biol., 22:161, 1980). The function of this response is unknown, and its relation to chemotaxis has not yet been examined. In the present paper, pharmacological inhibition of methylation is used to study the relation between methylation and chemotaxis.

Biological transmethylation requires S-adenosylmethionine (AdoMet) as a methyl donor, which is synthesized from methionine and ATP by the enzyme ATP:L-methionine S-adenosyltransferase (see Fig. 1). Intracellular AdoMet pools are usually small, i.e., 30 to 50 nmol/g (wet weight) (13, 14, 16, 27) and show a rapid turnover (complete in 1 to 10 min) (2, 9, 14). Therefore, agents which cause an inhibition of the biosynthesis of AdoMet induce a rapid decline of AdoMet levels and inhibition of methylation. The methionine analog cycloleucine (1-aminocyclopentanecarboxylic acid) has been shown to inhibit bacterial, fungal, and vertebrate ATP:L-methionine S-adenosyltransferases with  $K_i$  values between 2 and 6 mM (7, 23, 24, 42). Biological effects of this compound include inhibition of chemotaxis in mouse macrophages (1), a decrease of adaptation to chemotactic signals in the bacterium *Salmonella typhimurium* (2), and an inhibition of protein carboxyl methylation in human erythrocytes (16), indicating its suitability as an inhibitor of transmethylation in vivo.

As shown in Fig. 1, methylation gives rise to the formation of the end product S-adenosylhomocysteine (AdoHcy), a strong inhibitor of methyltransferases (6, 35). AdoHcy levels

are kept low by breakdown via the enzymes AdoHcy hydrolase and adenosine (Ado) deaminase. Agents which inhibit Ado catabolism will induce a rapid accumulation of AdoHcy, since the equilibrium of the AdoHcy hydrolase-catalyzed reaction favors condensation of Ado and L-homocysteine. For these reasons, any inhibition of Ado deaminase will be accompanied by a decrease of the rate of transmethylation.

Erythro-9-(2-hydroxy-3-nonyl)adenine (EHNA) is a well-known inhibitor of Ado deaminase with  $K_i$  values between 20 and 50 nM (6, 22, 39). Biological effects of this compound include blocking of transmethylation in several types of human and rat tissues (15, 21, 29, 35, 37), inhibition of chemotaxis, chemotactic polarization and histamine release in leukocytes (6, 28, 35, 36), and a decrease of immunoglobulin-induced receptor capping in human lymphocytes (3).

The antibiotic coformycin (3- $\beta$ -D-ribofuranosyl-6,7,8-trihydroimidazo[3,4-d][1,3]diazepin-8(R)-ol) is a transition state inhibitor of Ado deaminase with  $K_i$  values between 0.1 and 5 nM (5, 30). Like EHNA, coformycin has been shown to inhibit immunoglobulin-induced receptor capping (3).

The effect of Ado deaminase inhibitors on the rate of transmethylation can be increased by addition of L-homocysteine or its lipophilic analog L-homocysteine thiolactone (Hcy-TL), since the administration of these compounds will stimulate the accumulation of AdoHcy (Fig. 1).

In the present paper, the influence of the mentioned compounds on cell aggregation, chemotaxis, and protein carboxyl methylation of *D. discoideum* is examined. The results are presented as evidence for the involvement of methylation reactions in the process of chemotaxis.

## MATERIALS AND METHODS

**Chemicals.** L-[methyl- $^3$ H]methionine (3.15 TBq/mmol) and [8- $^3$ H]cAMP (0.9 TBq/mmol) were obtained from the Radiochemical Centre, Amersham, United Kingdom. cAMP was purchased from Boehringer Mannheim Biochemicals, Mannheim, Federal Republic of Germany. Coformycin was a

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product of Meiji Seika Kaisha, Tokyo, Japan. Cycloleucine, Hcy-TL, and snake venom (*Ophiophagus hannah*) were obtained from Sigma Chemical Co., St. Louis, Mo. EHNA was a generous gift from the Burroughs-Wellcome Laboratories, Research Triangle Park, N.C. Stock solutions of inhibitors ( $10^{-3}$  to  $10^{-1}$  M) were prepared in water and stored at  $-20^{\circ}\text{C}$  until used.

**Culture conditions.** *D. discoideum* NC-4(H) was used for all experiments. Cells were grown in association with *Escherichia coli* 281 on a solid medium (3.3 g of peptone, 3.3 g of glucose, 4.5 g of  $\text{KH}_2\text{PO}_4$ , 1.5 g of  $\text{Na}_2\text{HPO}_4 \cdot 2\text{H}_2\text{O}$ , 15 g of agar per liter) and were harvested in phosphate buffer (10 mM  $\text{Na}_2\text{HPO}_4 \cdot 2\text{H}_2\text{O}/\text{KH}_2\text{PO}_4$  [pH 6.5]). They were freed from bacteria by repeated centrifugation (three times at  $150 \times g$  for 4 min).

**cAMP binding and phosphodiesterase assays.** Binding of cAMP to the cell surface of aggregative amoebae was measured by the ammonium sulfate stabilization assay (44) at a [ $^3\text{H}$ ]-labeled cAMP concentration of 10 nM. Phosphodiesterase activity was assayed as described previously (45). The incubation time was 10 s.

**Cell aggregation and chemotaxis.** Cells were starved on nonnutrient agar (15 g of agar, 4.5 g of  $\text{KH}_2\text{PO}_4$ , 1.5 g of  $\text{Na}_2\text{HPO}_4 \cdot 2\text{H}_2\text{O}$  per liter) at a density of  $3 \times 10^5$  cells per  $\text{cm}^2$ . After 8 h cells were harvested, washed once with 10 mM  $\text{Na}_2\text{HPO}_4 \cdot 2\text{H}_2\text{O}/\text{KH}_2\text{PO}_4$  (pH 6.5), and suspended in the same buffer at a density of  $5 \times 10^6$  cells per ml. Small droplets (0.1  $\mu\text{l}$ ) of the suspension were deposited on hydrophobic agar plates (17) which contained inhibitors at the concentrations mentioned below. During preparation of the hydrophobic agar plates, stock solutions of inhibitors were added to the agar when it was still in its fluid state (temperature,  $60^{\circ}\text{C}$ ), and the inhibitor was distributed over the entire plate by gentle rotation of the petri dish.

The number of populations which showed an aggregation center was monitored during a 7-h period after deposition of the cells on hydrophobic agar. The first aggregation center was seen at ca. 1.5 h in control plates, whereas at 2.9 h 50% of the control populations showed aggregation.

Chemotaxis tests were started when cells had been on the hydrophobic agar for 1 h. Small drops (0.1  $\mu\text{l}$ ) of different cAMP solutions ( $10^{-9}$  to  $10^{-7}$  M) were deposited at a distance of about 300  $\mu\text{m}$  from the amoebal populations. The chemotactic response was monitored at 15-min intervals as described previously (17).

**Methylation assay.** Cells were starved in suspension for 5 h, harvested, and resuspended at a density of  $10^8$  cells per ml as described above. Conditioning was performed in siliconized glass beakers (15 ml) which were continuously shaken (200 rpm; Gyrotory shaker; New Brunswick Scientific Co., New Brunswick, N.J.). The amoebae were incubated with cycloheximide (250  $\mu\text{g}/\text{ml}$ ) and methylation inhibitors for 1 h, followed by an additional 30 min in the presence of L-[methyl- $^3\text{H}$ ]methionine (2  $\mu\text{Ci}/\text{ml}$ ). After conditioning, 100- $\mu\text{l}$  samples of suspension were added with a pipette into Eppendorf reaction vessels and continuously shaken by an Eppendorf mixer. Cells were stimulated with a  $10^{-6}$  M cAMP pulse (being the optimum concentration for the induction of a methylation response) (46) and lysed after different time intervals by addition of 10  $\mu\text{l}$  of 70%  $\text{HClO}_4$ . Perchlorate lysates were centrifuged ( $8,000 \times g$  for 5 min) and stored overnight at  $4^{\circ}\text{C}$ . Protein pellets were suspended in 50  $\mu\text{l}$  of a solution containing 0.6 M acetic acid, 5 M urea, 1% 2-mercaptoethanol, and 2% *N*-cetylpyridinium chloride by heating for 5 min at  $95^{\circ}\text{C}$ . Electrophoresis of proteins was performed in rod gels (85 by 5 mm) containing 5% acetic

acid, 1% *N,N,N',N'*-tetramethylethylenediamine (TEMED), 10% acrylamide, 0.25% *N,N'*-methylenebisacrylamide, 20% glycerol, 5 M urea, and 0.1% ammonium persulfate as described previously (10). Electrophoresis was continued at a current of 2 mA per gel until the marker protein, cytochrome *c*, had reached the bottom of the gel. After electrophoresis gels were washed, dried on filtration paper, and manually homogenized in small sections. Homogenized gels were incubated overnight in 15 ml of Instagel (Packard Instrument Co., Inc., Rockville, Md.), and protein-associated radioactivity was measured the next morning by liquid scintillation counting with automatic quench correction. Under these conditions, at least 85% of the measured radioactivity has been shown to be due to protein carboxyl methylation (46).

## RESULTS

**Influence of inhibitors on cAMP binding and phosphodiesterase activity.** The transmethylation inhibitors which act via inhibition of Ado deaminase activity (enzyme 5 in Fig. 1), i.e., EHNA and coformycin, are derivatives of Ado. Since Ado inhibits the binding of cAMP to its receptor (31, 43) we first evaluated the influence of the inhibitors on cAMP binding. Cycloleucine, Hcy-TL, and coformycin did not influence cAMP binding (Fig. 2). EHNA, however, inhibited cAMP binding at concentrations above 30  $\mu\text{M}$  (Fig. 2).

The proper function of phosphodiesterase is important to chemotaxis since a mutant which lacks the enzyme is incapable of cell aggregation (8). For this reason, we also evaluated the influence of inhibitors on phosphodiesterase activity. The activity of total (extracellular plus membrane-bound) phosphodiesterase proved to be not significantly affected by 25 mM cycloleucine, 1 mM Hcy-TL, 35  $\mu\text{M}$

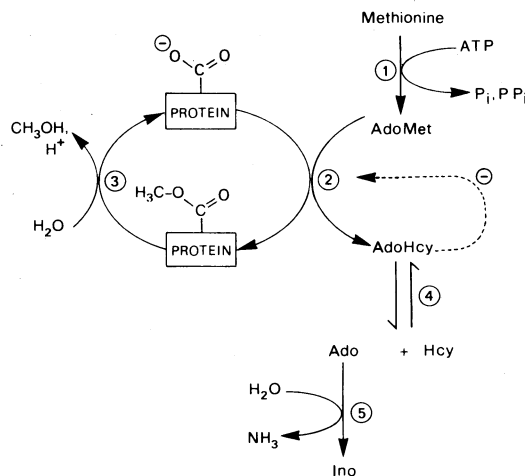


FIG. 1. Coupling of protein carboxyl methylation to nucleotide metabolism. Enzymes involved are: 1, ATP:L-methionine *S*-adenosyltransferase (EC 2.5.1.6); 2, protein *O*-methyltransferase (EC 2.1.1.24); 3, protein methyltransferase (not yet included in the Enzyme Commission list); 4, *S*-adenosylhomocysteine hydrolase (EC 3.3.1.1); 5, Ado deaminase (EC 3.5.4.4). Cycloleucine is an inhibitor of enzyme 1, causing a decline of the AdoMet level and concomitant inhibition of transmethylation. EHNA and coformycin are inhibitors of enzyme 5. Their administration results in an increase of Ado and AdoHcy levels, the latter compound being a strong inhibitor of methyltransferases. Since the addition of L-homocysteine (or its lipophilic analog, Hcy-TL) will result in an increased accumulation of AdoHcy, L-homocysteine can also be used as an inhibitor of transmethylation. Ino, Inosine.

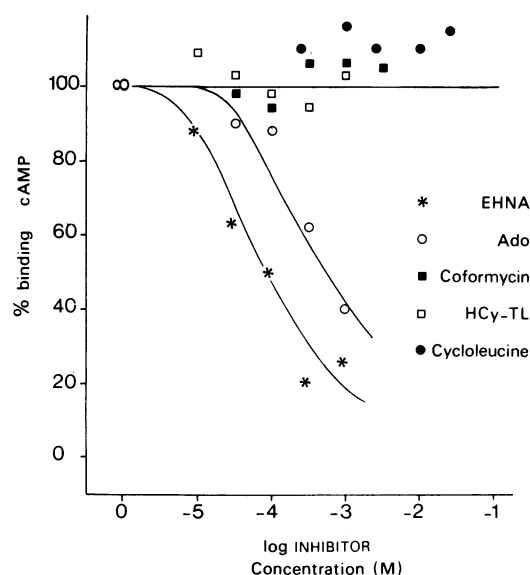


FIG. 2. Effect of inhibitors on cAMP binding to cell surface receptors. Binding of cAMP to the cell surface of aggregative amoebae was measured by the ammonium sulfate stabilization assay (44) at a [ $^3\text{H}$ ]-labeled cAMP concentration of 10 nM. Inhibitors were included in the assay at the concentrations shown in the figure.

coformycin, or 30  $\mu\text{M}$  EHNA (difference between control and experimental values less than 10%).

**Delay of cell aggregation.** Typical time curves for the influence of a methylation inhibitor on cell aggregation are shown in Fig. 3. At a dose of 0.35  $\mu\text{M}$ , coformycin did not induce a significant delay of aggregation, but a dose of 3.5  $\mu\text{M}$  was already highly effective, whereas at 35  $\mu\text{M}$  aggregation was totally inhibited during the 7-h period of observation. After 24 h, however, 100% aggregation was reached in all cases, and 100% development to fruiting bodies was reached within 48 h.

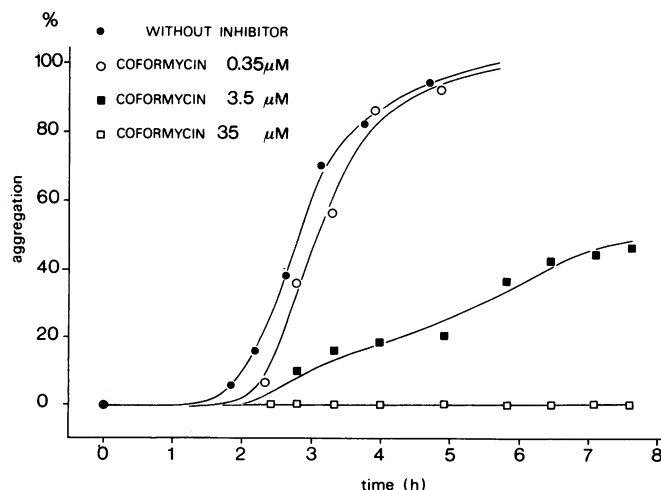


FIG. 3. Effect of coformycin on cell aggregation. Cell aggregation was monitored in small populations of *D. discoideum* which were deposited on hydrophobic agar after a previous 8-h period of starvation. Coformycin was dissolved in the agar at the concentrations shown in the figure. Each curve is based on the observation of 50 populations over a 7-h period.

TABLE 1. Effect of transmethylation inhibitors on cell aggregation in *D. discoideum*

Inhibitor	Dose (mM)	Time (h) <sup>a</sup>
Cycloleucine	0	2.9 $\pm$ 0.2
	0.25	2.6
	2.5	3.0
	25	>9.0
Hcy-TL	—	—
	0.01	3.0
	0.1	3.8
	1	6.2
Coformycin <sup>b</sup>	—	—
	0.35	3.1
	3.5	8.0
	35	>9.0

<sup>a</sup> Time taken to form aggregation centers in 50% of the population. All experimental values are based on the observation of 50 populations. The control value was determined from six independent observations, each based on 50 populations. Cell aggregation was monitored in small populations which had been deposited on hydrophobic agar plates after an 8-h period of starvation. Inhibitors were dissolved in the agar.

<sup>b</sup> Micromolar doses.

The effect of methylation inhibitors on aggregation time is summarized in Tables 1 and 2. The presence of cycloleucine (25 mM), Hcy-TL (1 mM), and coformycin (3.5 and 35  $\mu\text{M}$ ) in the agar caused a strong delay of aggregation ranging from 3 to more than 6 h. However, 100% aggregation was finally reached under all circumstances, and fruiting bodies were formed in all populations.

Low concentrations of EHNA, coformycin, or Hcy-TL delayed aggregation for several minutes, but combinations of an Ado deaminase inhibitor with Hcy-TL were able to increase aggregation time by more than 1 h (Table 2). Such a combination, moreover, induced a delay of aggregation which was greater than the sum of the effects of the individual components.

**Decrease of chemotactic sensitivity to cAMP.** Typical dose-response curves for the effect of the methylation inhibitors on cAMP-induced chemotaxis are shown in Fig. 4A. Incubation with coformycin resulted in a shift of the dose-response curves to higher cAMP concentrations, but the shape of the curves was not affected. Cycloleucine and Hcy-TL induced similar shifts of the dose-response curves. The effects of the inhibitors on the chemotactic activity of 10 nM cAMP is shown in Fig. 4B through D.

In the presence of Hcy-TL or coformycin, an additional effect on chemotaxis was observed. Normally, aggregative

TABLE 2. Effect of a combination of Ado deaminase inhibitors with Hcy-TL on aggregation of *D. discoideum*

Condition	Delay (h) <sup>a</sup>
25 nM EHNA	0.47
0.1 mM Hcy-TL	0.80
25 nM EHNA + 0.1 mM Hcy-TL	2.05
0.35 M Coformycin	0.15
0.1 mM Hcy-TL	0.40
0.35 M Coformycin + 0.1 mM Hcy-TL	1.15

<sup>a</sup> The delay of aggregation (in hours) with respect to a control (without inhibitor) obtained from the same batch of cells. All values are based on the observation of 50 populations. Cell aggregation was monitored in small populations which had been deposited on hydrophobic agar. Inhibitors were dissolved in the agar.

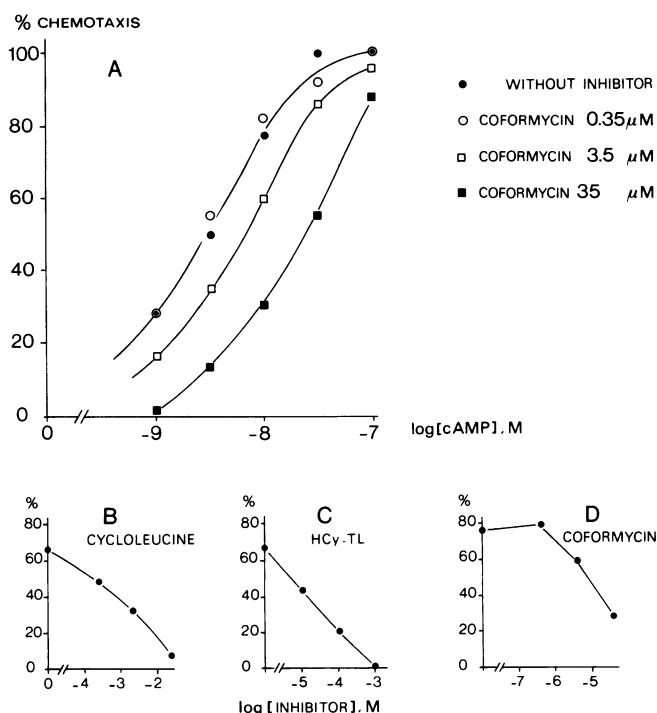


FIG. 4. Effect of inhibitors on chemotaxis to cAMP. Chemotaxis to different concentrations of cAMP was monitored in aggregative cells by the small population test as described previously (17). Inhibitors were dissolved in the hydrophobic agar, and the chemotactic stimulus was applied after 1 h of preincubation in the presence of the drugs. A, Influence of different concentrations of coformycin (0.35, 3.5, and 35  $\mu$ M) on chemotactic sensitivity of *D. discoideum*. Dose-response curves for cAMP chemotaxis are presented for a control and three concentrations of inhibitor. B through D, Influence of different concentrations of cycloleucine, Hcy-TL, and coformycin on chemotaxis of *D. discoideum* to 10 nM cAMP. Indicated are the percentages of the total number of populations showing a positive response to cAMP.

amoebae respond positively to cAMP-concentrations up to 1  $\mu$ M. cAMP in excess of 1  $\mu$ M induces a radial response, i.e., the amoebae crawl in all directions away from the center of the drop (18). In the presence of 1 mM Hcy-TL or 35  $\mu$ M coformycin, the threshold for this radial response was lowered from ca. 10 to ca. 0.1  $\mu$ M cAMP. Cycloleucine at 25 mM did not show this effect.

**Influence of inhibitors on methylation.** At the concentrations used in this study, the effect of inhibitors on basal protein carboxyl methylation (i.e., methylation in the absence of a chemotactic stimulus) was found to be highly variable. Basal methylation was either unaffected or increased, and the effects of inhibitors on basal methylation levels did not correlate with their influence on aggregation time or chemotaxis.

A dose of inhibitor which induces a significant delay of aggregation, however, was found to convert the normal positive response of methylation to cAMP into a negative one (Fig. 5, Table 3). A typical time curve for the effect of a methylation inhibitor on the response of protein carboxyl methylation is presented in Fig. 5. In the absence of inhibitor, oscillations are seen with maxima which are situated above the control level. However, in the presence of an inhibitor, the first transient positive peak is changed into a transient demethylation. Although the second peak is just

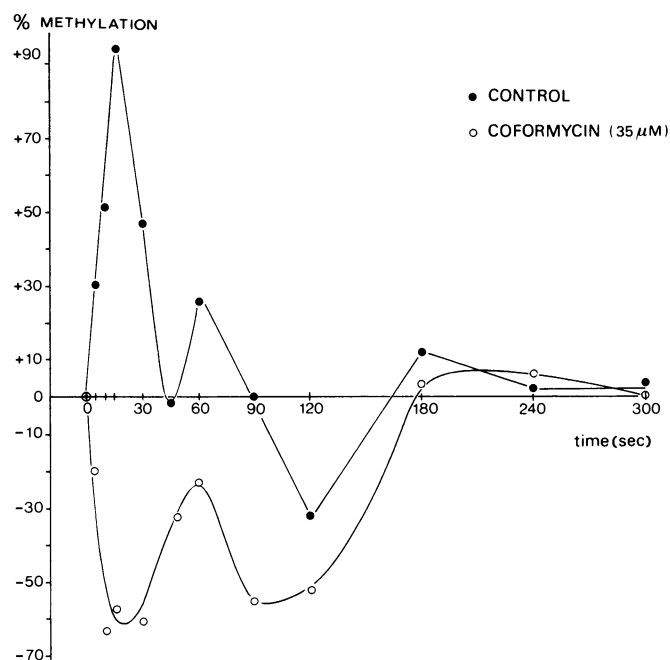


FIG. 5. Time course of methylation in the presence of coformycin. Cycloheximide-treated, L-[methyl- $^3$ H]methionine-labeled cells were stimulated with a 1  $\mu$ M cAMP pulse both in the presence and absence of 35  $\mu$ M coformycin. The reaction was stopped at different intervals after stimulus administration by the addition of a 0.1 vol of 70%  $\text{HClO}_4$ . Proteins were separated from phospholipid by electrophoresis on acetic acid-urea-containing polyacrylamide rod gels. Gels were washed and fragmented before incubation in scintillation fluid. After overnight incubation, radioactivity was measured by liquid scintillation counting with automatic quench correction.

visible, its maximum is far below the control value. The third maximum, occurring 3 to 5 min after stimulus administration, is hardly affected by the presence of the inhibitor. Similar patterns of inhibition were found in the presence of Hcy-TL (1 mM) or cycloleucine (25 mM). Although rapid, transient changes of methylation are blocked, slow changes can still occur, indicating incomplete inhibition of protein O-methyltransferase.

## DISCUSSION

The methylation inhibitors used in the present study do not influence the primary loci of cAMP recognition, i.e., the

TABLE 3. Correlation between inhibition of carboxylmethylation, cell aggregation, and chemotaxis

Compound	Function inhibited		
	Methylation <sup>a</sup>	Chemotaxis <sup>b</sup>	Aggregation <sup>c</sup>
Cycloleucine	0.8 mM	2.5 mM	4 mM
Hcy-TL	12 $\mu$ M	28 $\mu$ M	80 $\mu$ M
Coformycin	10 $\mu$ M	20 $\mu$ M	1 $\mu$ M

<sup>a</sup> Doses of inhibitor resulting in complete suppression of the initial positive response of methylation to cAMP. (The transition point on a dose-response curve in which the positive response of methylation to cAMP is changed to a negative one, i.e., the dose of inhibitor at which no response of methylation to cAMP is observed; see Fig. 5.)

<sup>b</sup> Doses of inhibitor resulting in 50% reduction of chemotactic response to 10 nM cAMP.

<sup>c</sup> Doses of inhibitor resulting in significant delay of aggregation (ca. 0.75 h).

cell surface receptors for cAMP and phosphodiesterase activity. Moreover, the inhibitors are not toxic to the cells. The influence of the inhibitors on the starvation process was investigated by incubation of the cells with drugs during the entire 10-h period of starvation. In these populations, we observed about the same delay of cell aggregation as in populations which were incubated with the inhibitors only during the final 1.5 h (data not shown). Furthermore, inhibitors did not dramatically affect cell differentiation, since normal fruiting bodies were finally formed in all experiments. The main targets of inhibition seem to be chemotaxis and cell aggregation, but not the acquisition of aggregation competence or cell differentiation.

A combination of an Ado deaminase inhibitor (EHNA or coformycin) and Hcy-TL induces a delay of cell aggregation which is greater than the sum of the delays caused by the individual components. This strongly suggests that the effect of the inhibitors is due to accumulation of AdoHcy with a concomitant inhibition of transmethylation (Fig. 1).

Several processes occur during cell aggregation, such as the initiation of an aggregation center, the release of the chemoattractant cAMP, the relay of the cAMP signal, positive chemotaxis to cAMP gradients, and the formation of cell-cell contacts. Methylation inhibitors may affect each of these processes. We observed that all inhibitors causing a delay of cell aggregation also decreased the chemotactic response towards cAMP. Inhibition of methylation via reduction of the AdoMet pool (i.e., by cycloleucine) resulted only in a shift of the dose-response curves to higher cAMP concentrations. Inhibition of methylation via accumulation of AdoHcy (i.e., by coformycin or Hcy-TL) resulted also in a qualitative diminution of the chemotactic response, since the response is no longer unidirectional. Apparently, cells still detect cAMP, but they are no longer able to analyze the chemotactic signal in a proper way.

The effects of methylation inhibitors on protein carboxyl methylation were unexpected. The inhibitors did not decrease basal protein methylation. In the absence of drugs, cAMP induces oscillations of methylation with three peaks reaching maxima at 15, 60, and 180 s after stimulation (Fig. 5). In the presence of inhibitor, the methylation level declines rapidly, so that the first peak, occurring after 15 s, is no longer observed. A remnant of the second peak, occurring 60 s after stimulus administration, is still visible, although its top is far below the control level. Methylation of the third maximum at 180 s is almost identical to that in control suspensions. Clearly, methylation inhibitors only depress the rapid transient methylation response without decreasing the slow response or the basal methylation level.

The oscillations of methylation are probably due to protein *O*-methyltransferase and protein methylesterase activity. The negative response in the presence of inhibitors (Fig. 5) suggests that cAMP stimulates the activity of both enzymes. Since the inversion of the methylation response could be correlated with the effectivity of the inhibitors to decrease chemotaxis (Fig. 5, Table 3), it may be hypothesized that a relationship exists between the rapid transient rise of protein carboxyl methylation and the chemotactic response to cAMP. However, other methylation reactions, such as phospholipid *N*-methylation, may also be involved since all AdoMet-requiring enzymes will be inhibited under the conditions of our experiments.

Effects of the inhibitors cycloleucine and Hcy-TL on methylation and chemotaxis correlated with those on cell aggregation (Table 3). However, much lower concentrations of coformycin were necessary to delay cell aggregation than

to inhibit methylation or chemotaxis (Table 3). Therefore, the effect of coformycin on cell aggregation seems not only to be caused by inhibition of cAMP chemotaxis but also by other mechanisms.

In bacteria, reversible methylation of membrane proteins has been shown to be essential for the proper processing of chemotactic signals (2, 20, 34, 38). When intracellular AdoMet levels are decreased (by methionine starvation of a methionine auxotrophic mutant or by inhibition of ATP:L-methionine *S*-adenosyltransferase with cycloleucine), chemotactic behavior is significantly affected. In normal cells, tumbling frequency is increased by the addition of a repellent and decreased on addition of an attractant. Since the cells adapt to a constant stimulus, prestimulus tumbling frequencies are resumed within a relatively short time period. When methylation is inhibited by lowering of the cellular AdoMet pool, bacteria remain able to change their tumbling frequency as a response to chemotactic agonists, but they fail to adapt. As a consequence of this, responses to a constant stimulus become permanent instead of being temporal (2, 20, 34). Methylation levels of certain gene products are responsive to levels of attractants and repellents in the medium; the period during which active methylation occurs is identical to the time it takes for the bacterium to adapt (20, 38). Mutant studies have shown that cells lacking protein *O*-methyltransferase are unable to adapt, whereas cells which lack the methyl acceptor proteins are unable to sense any chemotactic stimulus (34). Therefore, the methylated proteins seem to be part of the transduction chain leading to excitation, and carboxyl methylation itself is a regulatory process for the modification of cellular sensitivity and adaptation to constant stimuli.

In eucaryotic organisms research is much hampered by the extreme instability of the methyl groups, which are relatively stable only at low temperature and pH (10, 16; J. H. Nuske, Eur. J. Cell Biol. 22:161, 1980). Transient increases of carboxyl methylation on addition of chemoattractant were reported for rabbit neutrophils (32; R. F. O'Dea, O. H. Viveros, S. Aswanikumar, E. Schiffman, P. K. Chiang, G. L. Cantoni, and J. Axelrod, Fed. Proc. 37:1656, 1978) and cellular slime molds (27, 46; J. H. Nuske, Eur. J. Cell Biol. 22:161, 1980), but they could not be observed in human monocytes or guinea pig macrophages (35, 36, 40, 41). In neutrophils both protein *O*-methyltransferase and protein methylesterase are transiently activated on addition of a chemotactic stimulus, leading to a transient increase of the turnover of methyl groups (47), but such an increase does not occur in monocytes or macrophages (40). However, in all cell types studied thus far, chemotaxis is inhibited by drugs which block transmethylation (6, 35, 36, 41; O'Dea et al., Fed. Proc. 37:1656, 1978). Methylation seems to be involved in the initial transduction of the chemotactic signals rather than to be necessary for sustained directed migration since not only chemotaxis but also the rapidly occurring polarization of the cells after administration of a chemotactic stimulus is decreased by inhibitors of transmethylation (36).

In the present paper the chemoattractant cAMP is shown to induce transient increases of carboxyl methylation in *D. discoideum*. Both protein *O*-methyltransferase and protein methylesterase seem to be transiently activated, leading to transient increases of the turnover of methyl groups. Concentrations of drugs which cause a significant inhibition of the methylation response also cause a significant decrease of chemotaxis to 10 nM cAMP (Table 3). Chemotaxis is found to be not altogether blocked, but the dose-response curve for

the process is shifted to higher cAMP concentrations (Fig. 4A).

In future experiments, we intend to study the effects of inhibitors on cAMP relay, excitation, adaptation and de-adaptation of the cAMP-mediated cyclic GMP response (45), decreases in light scattering, and cAMP-induced calcium movements (27).

#### ACKNOWLEDGMENTS

We thank Theo Konijn and René de Wit for their critical reading of the manuscript. We are grateful to Albert Addink for the generous gift of a sample of coformycin. The help of Jan Herzberg in preparing the drawings is gratefully acknowledged.

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